Expert Opinion

Cyclin-dependent kinase inhibitors: a survey of the recent patent literature

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Since the mid-1990s cyclin-dependent kinase (CDK) inhibition has been the subject of an intense drug discovery effort in the pharmaceutical industry and in some academic institutions. Although only few compounds have at present progressed into human clinical trials, the prospect of finding safe agents useful in therapy, particularly in the cancer setting, is still positive. Some trends can be observed that witness the impact of recent findings in CDK basic biological research and technology advancements on the evolution of the patent literature on CDK inhibition. A patent literature review of small organic molecules as CDK inhibitors comprising the years 2001 – 2004 is presented here, as many of the major pharmaceutical companies have shown a continued effort in the field.

Keywords: cancer treatment, cell cycle, cyclin-dependent kinase (CDK), inhibitor

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1. Introduction

Uncontrolled cell growth and proliferation is a hallmark of all cancers. The cell cycle provides a model for describing how cells enter and accomplish a duplication round [1]. Within this model, upon application of an appropriate stimulus, such as light or growth factors, cells can undergo a number of biochemical processes that guide them from a quiescent state (G0) into a gap phase (G1) where the molecular machinery for the progression into DNA synthesis phase (S) is prepared. Following DNA duplication a second gap phase (G2) occurs prior to cell mitosis (M) and duplication. At this point, after integrating the internal status with external stimuli, cells can go back into the G0 state, or re-engage into a new cell cycle. Cyclin-dependent kinases (CDKs) are key regulators of the cell cycle progression. Complexes between CDK4/cyclin D are important at early G1, and CDK2/cyclin E and CDK2/cyclin A are key players for, respectively, overcoming the so-called restriction point where cells commit for another duplication round, and for accomplishing a successful S phase. CDK1/cyclin B is involved later on in the cycle when cells undergo mitosis. Early evidence that blocking CDK activity (and in particular CDK2) results in tumour cell antiproliferative activity and, possibly, selective apoptosis versus normal cells, triggered several research projects in the pharmaceutical industry and academia, aimed at finding selective or mixed CDK4, CDK2 or CDK1 inhibitors. Flavopiridol (Figure 1) was the first CDK inhibitor to enter clinical trials and it has also been a common reference standard in the field [2]. Other compounds are reported to have entered human clinical trials (Figure 1) [3], but conclusive data about the efficacy of CDK inhibitors in a clinical setting are still awaited. From a basic research perspective and target validation viewpoint, recent results have challenged the paramount role of CDKs in tumour cell proliferation [4,5], even if results obtained by methods such as gene knockouts and siRNA may not be directly comparable to enzyme inhibition with a small organic molecule. The different mode of action and the established and putative

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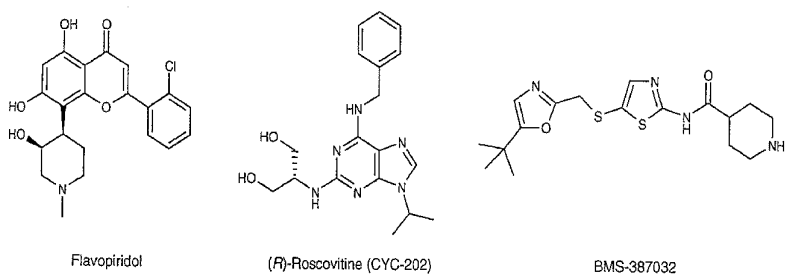


Figure 1. Disclosed CDK inhibitor clinical compounds.

applications of CDK inhibitors have been recently reviewed [6]. Beyond the established functions of CDK1, CDK2 and CDK4/CDK6, other CDKs have been proposed to play a role in cell cycle progression (CDK3/cyclin T) [7] and in transcription or other physiological and patho-physiological mechanisms (CDK7/cyclin H, CDK8/cyclin C and CDK9/cyclin T) [8,9]. CDK5/p25(p35) has been recently reviewed as a target in the CNS therapeutic area [10]. In any case, research on CDK inhibitors has been relentless, as witnessed by ~ 200 patents dealing with small organic molecules in the period 2001 – 2004. The scope of this review is to provide a survey of the most recent patent literature on the subject (2001 – 2004), and to highlight trends that emerge from this patent analysis.

2. New cyclin-dependent kinase inhibitors in the patent literature

As many of the pharmaceutical companies engaged in this field have been working on more than one chemical class, subchapters of the review refer to a specific company activity. It is important to note that inhibition data reported throughout this review may be influenced by several factors that may not be specified in the documents, including, but not limited to, solubility of the compounds, K_m of ATP for different CDKs and/or crossreacting kinases, biochemical assay type (e.g., SPA, Multiscreen) and modalities (e.g., dissolution, dilution), and nature and purity of the enzymes. For this reason, comparisons among different inhibitor classes from different companies should always be made with caution.

2.1 AstraZeneca

AstraZeneca (AZ) has been very active in the CDK arena during the last few years, in particular building upon its expertise in the pyrimidine field. A total of ninety-eight compounds combining bicyclic heterocyclic systems, such as imidazo[1,2-a]pyridine or pyrazolo[2,3-a]pyridine with a 2-aminopyrimidine core scaffold are claimed in a patent application [101]. Several nanomolar compounds belonging to this series have recently been published [11]. An example of these compounds is given by compound 1 (IC_{50} against CDK2/cyclin E = 5 nM; IC_{50} against MCF-7 tumour cell proliferation = 70 nM) (Figure 2). A cluster of four patents dealing with structurally simpler 2-aminopyrimidines subsequently appeared [102-105]. Compounds of [102] are anticancer molecules targeted at CDK4/cyclin D1 (primarily) and human focal adhesion kinase 3 (FAK-3). Compound 2 is reported in the patent with an IC_{50} of 0.366 µM against CDK4/cyclin D1 (Figure 2). The 2,4-diaminopyrimidine nucleus is a common substructure for compounds claimed in [103-105]. Again, a nonoptimised CDK2/cyclin E activity is reported for a compound of [103] (3; IC_{50} = 0.347 µM). Related 2,4-diaminopyrimidines are reported in [106]: a common feature to this subclass is the presence of a 5-cyano group on the 2,4-diaminopyrimidine kernel. A compound (4) with an IC_{50} value of 17 nM against CDK2/cyclin E is specifically claimed. A further 132 compounds belonging to the same subclass, but with different substituents at position 4 of the pyrimidine ring, are claimed as (preferentially) CDK2 inhibitors in other patent applications [107,108]. As a follow-up of the same main class expansion, 5-imidazol-2-yl-2-anilinopyrimidines have also been disclosed [109]. Finally, patents dealing with 4-(imidazol-5-yl)-2-(4-sulfoanilino)pyrimidines (e.g., 5) have been more recently disclosed [110-114]. Common features in AZ patents in this field are that: i) they all deal with compounds derived from a basic 2-pyrimidine ring; ii) a thorough description of the assay and the production of protein components is given; iii) solution-phase chemistry is detailed and compounds are characterised by both ^{1}H-NMR and MS.

2.2 Aventis

Aventis has pioneered the field with flavopiridol that, although endowed with other activities in addition to CDK inhibition, still is a frequently used reference compound in

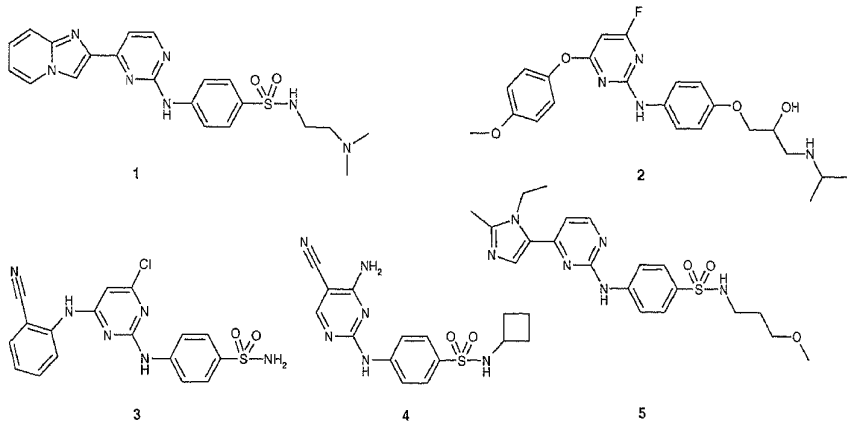


Figure 2. CDK inhibitors from AstraZeneca.

the field [2]. Not surprisingly, some patenting activity was thus devoted at covering the flavopiridol franchise with new solvates [115], polymorphs [116] and novel derivatives [117].

Aventis has also been active on different chemical classes such as *N*-acyl and *N*-sulfonyl-aminopurines [118] (Figure 3). In [118], 211 specifically claimed compounds are described. Cellular and early ADME data for a limited number of compounds are provided and IC_{50} values against tumour cell lines, such as colon Colo-205, prostate PC3 and leukaemia HL-60, are in the nanomolar range. Similar compounds are claimed in a separate patent application [119]. In this case, compounds similar to those claimed in [118] are stated to be antimicrobial agents, specifically inhibitors of *Candida albicans*.

Along the lines that led to the discovery of flavopiridol, natural compounds, such as klainetin A and B [120] (Figure 3), are claimed as CDK inhibitors useful for treating tumours, although the reported IC_{50} values against CDK2 and CDK4 are only micromolar (6.04 and 1.14 µM, respectively). Again natural products (caloporoside derivatives) are claimed in [121].

Only four compounds are exemplified in a very focused patent on oxindole (Figure 3) CDK1 inhibitors [122]. The specified compound showed an IC_{50} value of 0.28 µM for inhibition of colon HCT-116 cells.

More than 200 substituted benzimidazole compounds (e.g., 6, Figure 3) are claimed to be CDK2/cyclin E and CDK4/cyclin D inhibitor, some at nanomolar concentrations [123]. A total of 232 indazoles are specifically claimed in an Aventis patent, which indicates kinase platform development; several kinases with relevance in cancer are described. A specified compound (7, Figure 3) exhibited 93 – 100% inhibition of FAK, KDR, Aurora2, Src and CDK2 at 10 µM [124]. The same trend towards a multikinase (including CDKs) characterisation is visible in a further patent dealing with new purine derivatives [125] and in a document concerning substituted pyridazinones [126]. In general, Aventis patents in the CDK2 field are diversified across different templates without neglecting natural product-derived compounds.

2.3 Bristol-Myers Squibb Group

2.3.1 Bristol-Myers Squibb

Bristol-Myers Squibb (BMS) has been patenting in the CDK field since 1998. Two main classes have been developed over the years; the pyrazolo[3,4-b]pyridines and the 2-aminothiazoles; the latter class being seemingly the most important one. From this chemotype, a Phase I clinical candidate emerged (BMS-387032, 8, Figure 4) [12]. Patenting activity has been consistent in the last few years, mainly directed at consolidating the coverage of the 2-aminothiazole class, in particular building a strong patent case around the clinical compound. To this end, several continuation-in-parts and follow-ups of the originative 2-aminothiazole patent were filed [127-132]. Reference [127] is deemed as particularly important because it claims at least 10 different salt forms of the clinical candidate. As many as 735 compounds claimed [129] and a description of a solid-phase synthesis methodology are indications that extensive parallel synthesis has been performed on this compound class. A patent covering similar compounds, but with a 3-aminopyrazole core heterocycle (e.g., 9), was also filed [133]. Patenting around the pyrazolo[3,4-b]pyridine class [13] (e.g., 10) has been less prolific but still is an important part of the

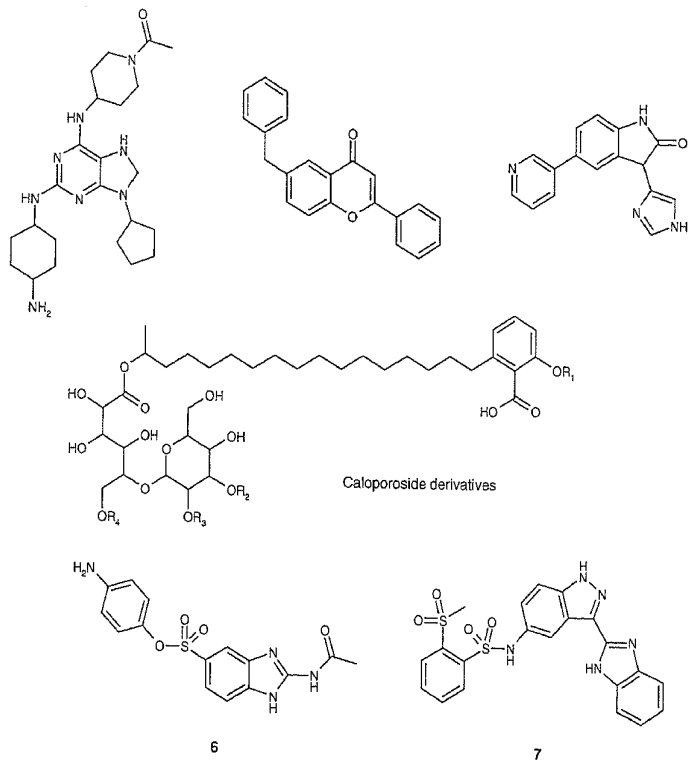


Figure 3. CDK inhibitors from Aventis.

BMS' CDK effort [134]. More recently, general patents claiming inhibitors of different kinases, encompassing also CDKs, were filed [135,136]. Although [135] and [136] should deal with novel benzimidazol-2-yl-1H-pyridin-2-ones tyrosine kinase inhibitors like compound 11, CDKs are also contemplated among the possible targets. An assay for CDK2/cyclin E is reported and > 600 compounds are specifically claimed. Once again, this is a compound class that, depending on the decorations applied onto the base scaffold, can provide different kinase selective inhibitors. Reference [137] describes more selected compounds in which a second ring is fused on the pyridin-2-one heterocycle, as in compound 12.

2.3.2 DuPont Pharmaceuticals

The activity of the former DuPont group working in the CDK field is shown by several patent applications that appeared throughout 2002 – 2003 [138-142]. Document [138] is certainly a central patent describing 1776 indeno[1,2-c]pyrazole semicarbazides as generic CDK/cyclin inhibitors. Although no specific activities are indicated some compounds of the patents are believed to be potent CDK2/cyclin E inhibitors in analogy to compounds of the same general class published elsewhere [14]. Selected derivatives with a 3-(2,4-dimethylthiazol-5-yl) groups are claimed in [139] (e.g., 13). Reference [140] deals with lead optimization compounds of the same class, such as 14, all of them bearing solubilising moieties.

2.4 Pfizer Group

The Pfizer Group, through its own patent activity and that one of the companies acquired in the last few years, can boast a strong franchise in the CDK field. At least 25 patents have been published in the 2001 – 2004 timeframe, dealing with CDKs, from companies that now constitute the Pfizer Group. For the sake of clarity, a patent analysis is done for each subcompany that originated the patents.

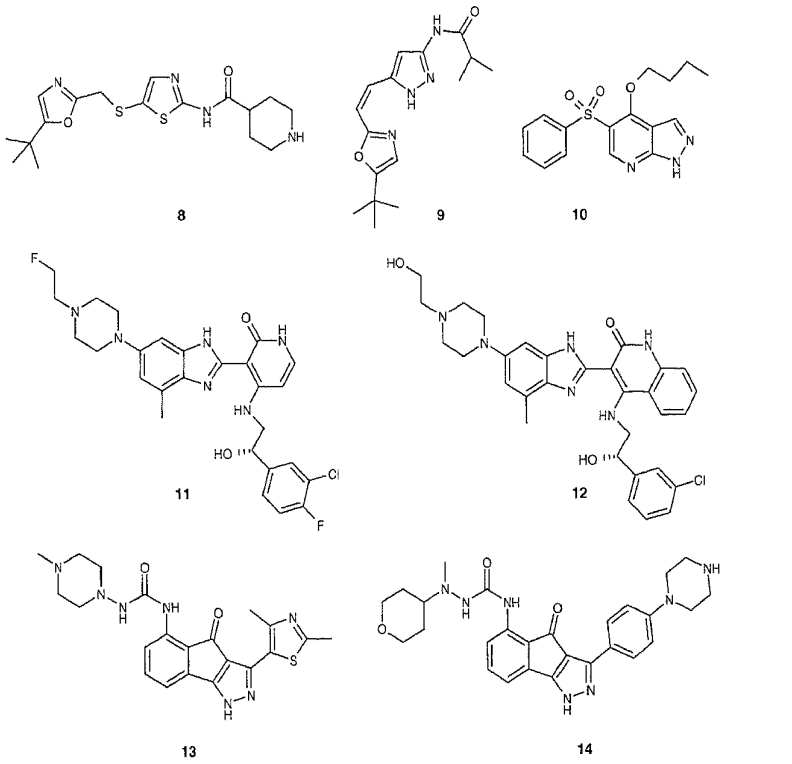


Figure 4. CDK inhibitors from Bristol-Myers Squibb.

2.4.1 *Pfizer*

Pfizer patented new imidazole derivatives [143] (e.g., **15**, Figure 5) as CDK5 and GSK-3β inhibitors. Due to the nature of the targets, the main indications sought are therefore neurodegeneration and metabolic diseases. A total of 28 compounds are exemplified and fully characterised by ^{1}H-NMR and MS. No specific biological data are provided. Along the same lines, pyrazole derivatives (**16**) were claimed [144], this time as generic protein kinase inhibitors including CDKs. In this case, 153 compounds were specifically claimed with 71 compounds fully characterised by ^{1}H- and ^{13}C-NMR and MS. Finally, 2-aminothiazoles are claimed also as CDK2 and CDK5 inhibitors in a patent application [145]. A selection of these compounds appeared in a recently published paper [15]. A CDK2/CDK5 selectivity ratio of 12.0 (CDK2-IC_{50}/CDK5/IC_{50}) was attained with compound **17**.

2.4.2 *Agouron*

Thiazoles, pyrazoles and indazoles are the heterocyclic nuclei around which Agouron based its work in the CDK inhibition field. In 2001, patent applications claiming indazoles [146] and pyrazoles [147] were filed. In the former case, compounds, such as **18** (Figure 6), are described with a detailed biological characterization: K_i values or percentages of inhibition at 1 μM are reported for inhibition of CDK4/cyclin D, CDK2/cyclin A, CHK1, VEGFR-2, LCK, FGFR and FAK. For selected compounds, cellular activity against colon tumour HCT116 cells with both IC_{50} and IC_{90} values is indicated. Compound **18** is a very potent CDK2/cyclin A inhibitor (K_i = 0.25 nM), less so but still notably active against CDK4/cyclin D (K_i = 14 nM). Its IC_{50} and IC_{90} values on HCT116 cells were 90 and 220 nM, respectively. A patent application [147] provides 3-aminopyrazoles, such as compound **19** (Figure 6),

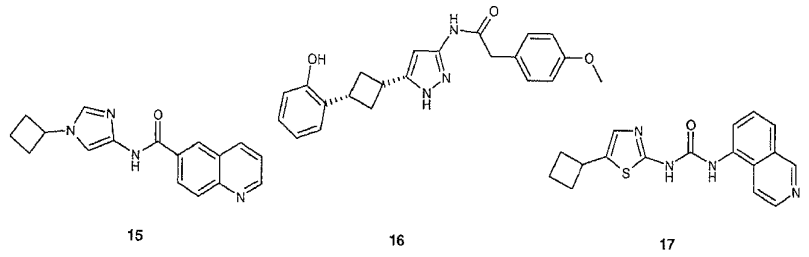


Figure 5. CDK inhibitors from Pfizer.

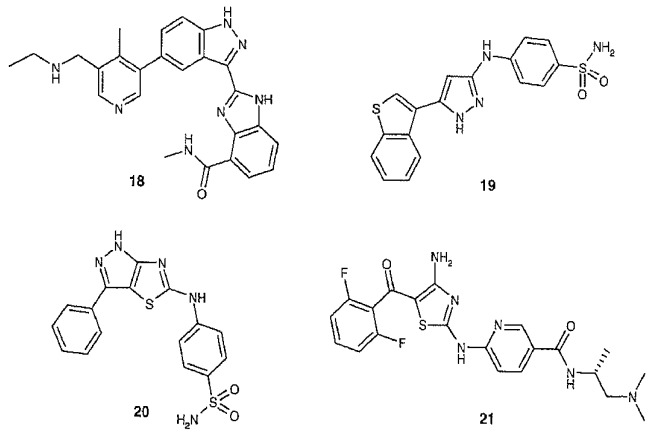


Figure 6. CDK inhibitors from Agouron.

which shows some selectivity for CDK2 with a CDK4 (1.6 μM)/CDK2 (0.062 μM) ratio of 25.8. Again, cellular activity is reported for compound **19** though a less potent one than for the indazoles (IC_{50} = 9.5 μM against HCT116). More recently, pyrazolo[3,4-d]thiazoles such as compound **20** (Figure 6) have been reported [148]. Less biological information than before is provided. Compound **20** is the most potent CDK2/cyclin A inhibitor reported with an IC_{50} value of 34 nM (390 nM for CDK4/cyclin D). Finally, tri-substituted 2-aminothiazoles (e.g, **21**, Figure 6) are the subject of three very recent patent applications [149-151]. As an example, compound **21** is an extremely potent CDK4/cyclin D inhibitor (IC_{50} = 0.61 nM), endowed with CDK2/cyclin A activity (IC_{50}: 4.4 nM), and IC_{50} and IC_{90} values against HCT116 cells of 2.6 and 5.7 nM, respectively.

2.4.3 *Warner-Lambert*

Warner-Lambert (Parke-Davis) spearheaded the efforts in the field of protein kinase inhibition during the 1990s. It is therefore not surprising that the patent activity around CDKs is relevant until its acquisition by Pfizer. In particular, at least five patents in 2001 aim at covering different classes of CDK inhibitors [152-156]. In the first patent application pteridinones are claimed as CDK inhibitors, although assays and data for other kinases (Wee, PDGFR, FGFR, c-Src) are also reported. Compound **22** (Figure 7) is a nicely selective CDK4/cyclin D inhibitor (IC_{50} = 7 nM vs. 750, 180, 610 against CDK1/cyclin B, CDk2/cyclin A and CDK2/cyclin E, respectively). From the same patent, compound **23** (Figure 7) is instead a potent c-Src inhibitor (IC_{50} = 15 nM) with some activity on Wee and PDGFR (subtype not specified) (IC_{50} = 860 and

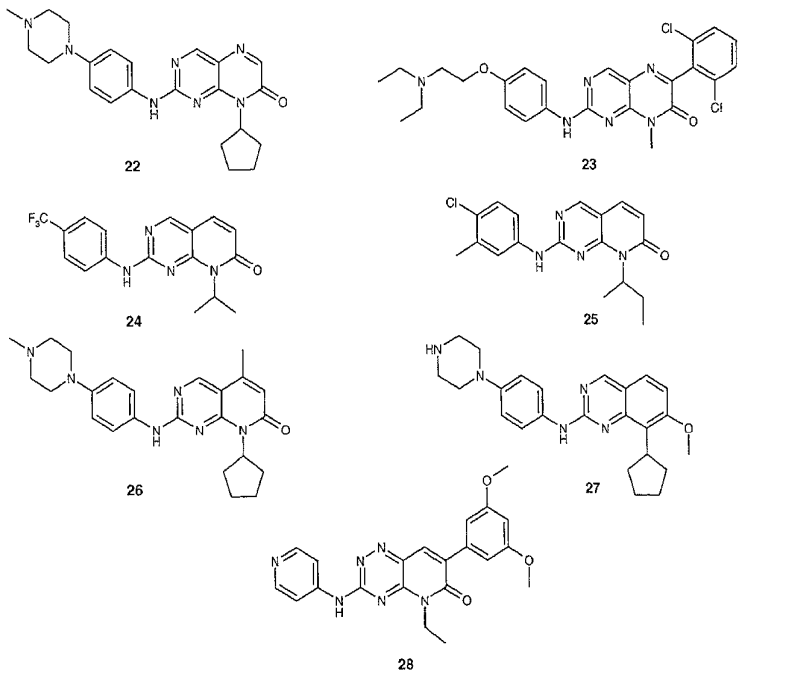


Figure 7. CDK inhibitors from Warner-Lambert.

700 nM, respectively). Analytical data for many compounds are thoroughly reported with melting points, elemental analyses, ^{1}H-NMR, and, sometimes, ^{13}C-NMR. Pyrido[2,3-d]pyrimidines and pyridopyrimidinones are clamed in [153] and [154], this time more specifically as CDK inhibitors. Reference [154] is a noteworthy patent application in that biochemical inhibitions against CDK4/cyclin D, CDK2/cyclin E, CDK2/cyclin A, CDK4/cyclin B and CDK5 (in a HTS format) are provided for 621 compounds, thus rendering a beautiful structure–activity relationship (SAR) landscape (if data can be quantitatively compared) for inter-CDK selectivity. As an example, compound 24 (Figure 7) shows some selectivity for CDK2/cyclin A (IC_{50} = 75 nM) versus CDK2/cyclin E (IC_{50} = 710 nM), whilst a similar compound (25) looks more selective for CDK5 (IC_{50} = 25 nM) as compared with CDK1/B, 2/E, 2/A and 4/D (IC_{50} = 130, 110, 310 and 130 nM, respectively). 5-Alkylpyrido[2,3-d]pyrimidines are selected compounds in [155]. In this document a compound (26) is characterised for its metabolic stability evaluated in human liver microsomes (HLM) and given as the times in minutes ($t_{1/2}$) required for half of the parent compound to disappear after being added to a HLM homogluate (85 min for 26 with a clearance of 25.5 ml/min/kg). Compound 26 is a potent and selective CDK4/cyclin D, inhibitor with an IC_{50} value of 7 nM (> 5 µM for CDK1/B, CDK2/A, CDK2/E; 1.077 µM for FGFR). The full exploration of the fused pyrimidine chemotype is documented by [156]: quinazolines are here reported with a preferential CDK4/cyclin D inhibition (e.g., 27; IC_{50} = 1 vs. 28, 132 and 250 nM for, respectively, CDK2/A, CDK1/B, and CDK2/E). Pyridotriazines and pyridopyridazines are finally claimed in [157]: for compound 28 (CDK inhibition data are not reported although assays are described) the most potent kinase activity is against VEGFR-2 with an IC_{50} value of 350 nM. Various mono- and bicyclic pyrimidines bearing a 2-aminopyridine substitution have also been recently claimed in [158] as CDK4/cyclin D inhibitors.

2.4.4 Pharmacia

Pharmacia has published several patents dealing with CDK inhibition; the most developed chemotypes being 2-aminothiazoles

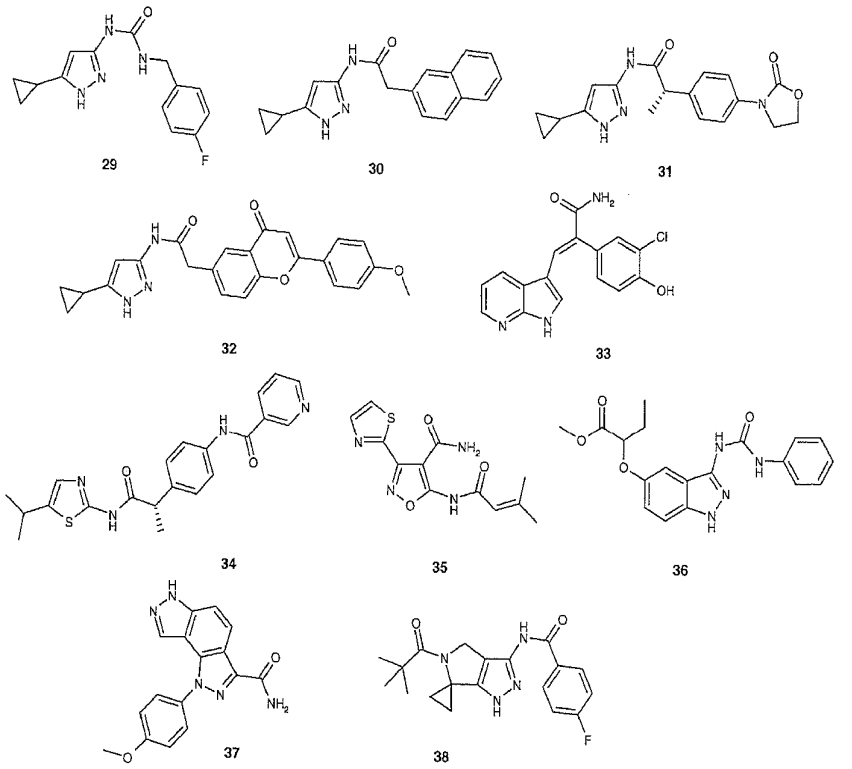
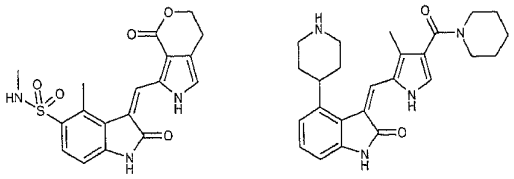


Figure 8. CDK inhibitors from Pharmacia.

and 3-aminopyrazoles, also fused to form bicyclic and tricyclic systems. 3-Aminopyrazolylureas and amides are the subject of two patent applications [159,160]. The latter describes 548 3-aminopyrazolylamides (an indication that parallel synthesis has been performed in the series) with a protocol for CDK2/cyclin A inhibition assay, but without specific biological data; compounds 29 and 30 (Figure 8) exemplify the series. Data on several 3-aminopyrazolylamide compounds are reported in a recent paper [16]. Selected 3-aminopyrazoles are the object of [161], and are represented by compound 31 (Figure 8). Another document [162] deals with 3-aminopyrazole derivatives bearing a chromenyl moiety on the 3-acetamido function (e.g., 32). Over 500 azaindoles (pyrrolo[2,3-b]pyridines) are claimed in a patent case [163]. Here, an enlarged panel of 10 kinase assays including CDKs is reported. Compound 33 exemplifies this class. A selection patent [164] of pre-2001 documents concerns 5-isopropyl-2-aminothiazoles with a suitably substituted 2-phenylacetamido group. Compound 34 is an example of the 40 compounds claimed in this patent. From a chemically unrelated class, the trisubstituted isoxazole compound 35 is an example of a library of ~ 1400 compounds [165]. A solid-phase synthesis process is also claimed for this class. Within a broader kinase effort comprising the search for CDK inhibitors, > 500 5-substituted indazoles are claimed [166], of which compound 36 is reported as an example. Benzodipyrazoles like 37 are described in a 2003 patent application [167]. Although biological data are not provided in the patent application, a paper dealing with these compounds has been published [17]. More recently, 3,5,6-substituted pyrrolo[3,4-c]pyrazoles [168] have been reported, represented by compound 38. It is apparent that



39 40

Figure 9. CDK inhibitors from Sugen.

within the former Pharmacia CDK effort, the choice was made to work around different chemotypes to overcome possible class-specific issues. Also, the variety of kinase assays described in the more recent patents witnesses a broader kinase effort, not limited to CDK inhibition.

2.4.5 Sugen

In the stream of patents published around the 2-indolinone (oxindole) class, Sugen filed two patent applications that are more specific for CDK inhibitors (Figure 9) [169,170]. In the former document, 3-pyrrolyllactone-2-indolinones are described as CDK2/cyclin A inhibitors, displaying selectivity over FLK, EGFR, and PDGFR. For example, compound 39 has an IC_{50} value against CDK2/cyclin A of 5.5 nM. Biological data are indicated for 58 compounds and the biology section of the patent gives a thorough description of biochemical, cellular, and even *in vivo* assessment methods. Compound 40 is an example taken from [170], the compound being a dual CDK2/A-FLK inhibitor (IC_{50} = 20 and 70 nM, respectively). A detailed study on a specific compound of these patents has been published [18].

2.5 Schering-Plough/Pharmacopeia

Bicyclic heterocycles, such as pyrrolopyrimidines, pyrrolopyridines and imidazopyrazines were disclosed in a series of recent patent showing a joint effort of Schering Corp. and Pharmacopeia in the CDK inhibition field (Figure 10) [171-178]. In particular, pyrrolopyrimidines were the subject of five patents applications dealing with different subclasses [171-175]. Compounds 41 – 45 exemplify these subclasses. Reference [166] is apparently central to this effort listing > 1000 compounds. Compound 41 is the most potent in a CDK2/Cyclin E assay with an IC_{50} value of 3 nM. Related patents (in the name of Schering Corp. only) were published shortly after [176-178] and are represented by compounds 46 – 48 (Figure 10). The groups used to decorate the central heterobicycle are very similar to those one reported in the previous pyrazolopyrimidine patents.

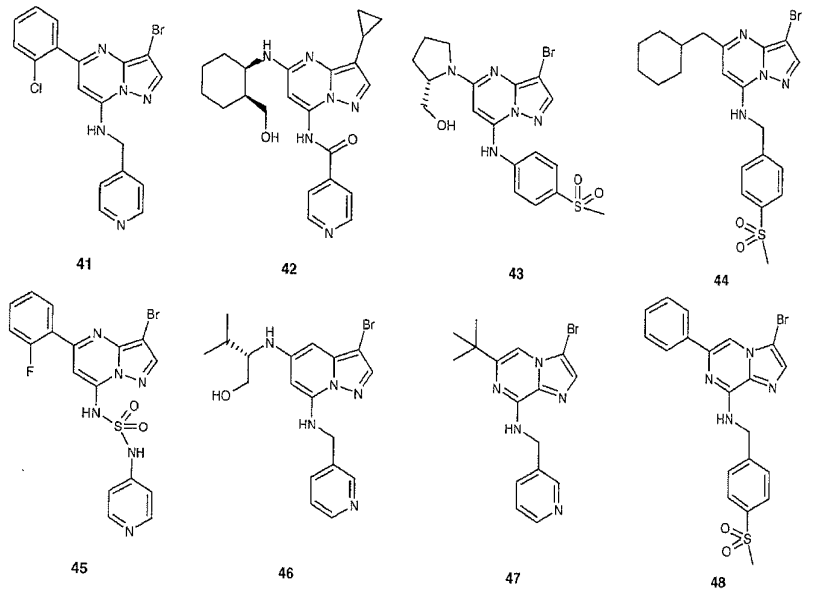
2.6 Vertex

Vertex has been very active in the kinase inhibition field, including CDK, in the last few years, as highlighted by the number of patent applications filed.

A cluster of three patents having the pyrazole nucleus as core structure (e.g., 49, 50, 51, Figure 11) was disclosed [179-181]. Compounds of these patents are preferentially claimed as GSK-3 and Aurora inhibitors, but CDK2 (and Akt) inhibition was also assessed. No biological data are presented for the specifically claimed compounds. A further 286 compounds belonging to the same subclass were claimed as CDK2, Aurora2, GSK-3, Src, ERK-2 and Akt inhibitors [182]. An example is given by compound 52 (Figure 11), which displayed a K_i value of < 0.1 µM against CDK2 [182]. The same trend towards a multikinase (including CDK2) characterisation is visible in two subsequent patents [183-184]. Compounds of [183] (e.g., 53) were disclosed as CDK2, ERK-2, GSK-3, Aurora, or Lck inhibitors and > 200 aryl heterocyclic derivatives (e.g., compound 54) are claimed to be JNK3, GSK-3, CDK2, Lck, and Src kinase inhibitors [184]. Only 13 compounds of type 55 (Figure 11) were disclosed in a patent on heterocyclic derivatives [185] CAK (CDK-activating kinase) inhibitors. The compounds are claimed to act as antimicrobial agents. CDK-activating kinase (CAK or CDK7) was isolated from *C. Albicans* and the activity measured by monitoring the phosphorylation of human CDK2 with [^{33}P]-ATP; assay details are given. A total of 12 compounds had K_i values in the range 0.06 – 2.4 µM, the specified compound 55 being the most potent. Contrary to the other patents, no syntheses are presented in [185].

Novel pyrazolone derivatives were claimed in particular as GSK-3, Aurora2 and CDK2 inhibitors [186]. An example is compound 56 which inhibits CDK2 activity by 87% at 2 µM. Over 70 novel pyrrole derivatives of type 57 (Figure 11) are specifically claimed as ERK2, CDK2, Aurora2, GSK-3, AKT3 or ROCK inhibitors in a patent application [187]. No biological data are presented.

A further three compounds belonging to the pyrazole subclass were disclosed in a patent application [188]. The



41 42 43 44

45 46 47 48

Figure 10. CDK inhibitors from Schering-Plough/Pharmacopeia.

compounds (e.g., 58, Figure 11) are stated to inhibit JAK3 and CDK2, but no specific data are reported.

2,4-Disubstituted pyrimidine is a common substructure in a cluster of four patents [189,191-193]. Compounds of [189] (e.g., 59) are claimed as protein kinase inhibitors (e.g., CDK2, ERK2, AKT3, GSK-3, p70s6k, PDK1, Aurora2, ROCK, Src, Syk, ZAP-70, JNK3, JAK3, TEC, LCK and Flt3 inhibitors) but no specific data are presented. About 100 pyrimidine derivatives of type 60 (Figure 11) are claimed individually to be JAK3, JNK3, CDK2 (K_i < 1.0 µM for the specified compound 60) and ZAP-70 inhibitors [191]. In two further patents [192,193], compounds belonging to the same subclass (61 and 62, Figure 11) were disclosed. These compounds are stated to be useful as inhibitors of JAK3, CDK2, JNK3, Syk and GSK-3 (K_i < 0.1 µM) [192] and of JAK, JNK, CDK2 and ZAP-70 (K_i < 1.0 µM) [193], respectively.

A multikinase activity was also claimed for compounds disclosed in other patents [190,194-196]. New indazolinone derivatives (e.g., compound 63) were claimed to be useful generally as protein kinase inhibitors, particularly as inhibitors of PRAK, GSK-3, ERK2, CDK2 (K_i < 1.0 µM for the specified compound 63), MK2, Src, Syk and Aurora2 [190]. Compounds of [194] (e.g., compound 64) were prepared as inhibitors of CDK, FLT-3, FMS, c-KIT, PDGFR, JAK, AGC subfamily, GSK, Src, ROCK or Syk. Again, no specific biological data are presented.

Compound 65 (Figure 11) is an example of new triazaindolizine derivatives, claimed in [195]. These compounds are stated to be active particularly against PIM-1, CDK2, GSK-3 and Src mammalian protein kinases.

As a follow-up of the same subclass expansion [183,187], novel heteroaryl substituted pyrroles (e.g., compound 66) have also been disclosed as CDK2, ERK-2, GSK-3 and PKA inhibitors [196].

Common features in all Vertex patents in the field are that: i) Vertex patents are rather diversified across different templates; ii) a kinase platform development is evident, as several kinases are described; and iii) kinases with relevance not only in cancer but also in other therapeutic area (e.g., metabolic, cardiovascular diseases, inflammation, infection, CNS, antimicrobial) are taken into consideration.

2.7 Schering AG

Schering AG has been progressively more active in the CDKs field in the last few years and its patenting activity spans across different templates (Figure 12).

A cluster of five patents dealing with indirubin derivatives was disclosed [197-201]. Compounds of [197] are claimed to act

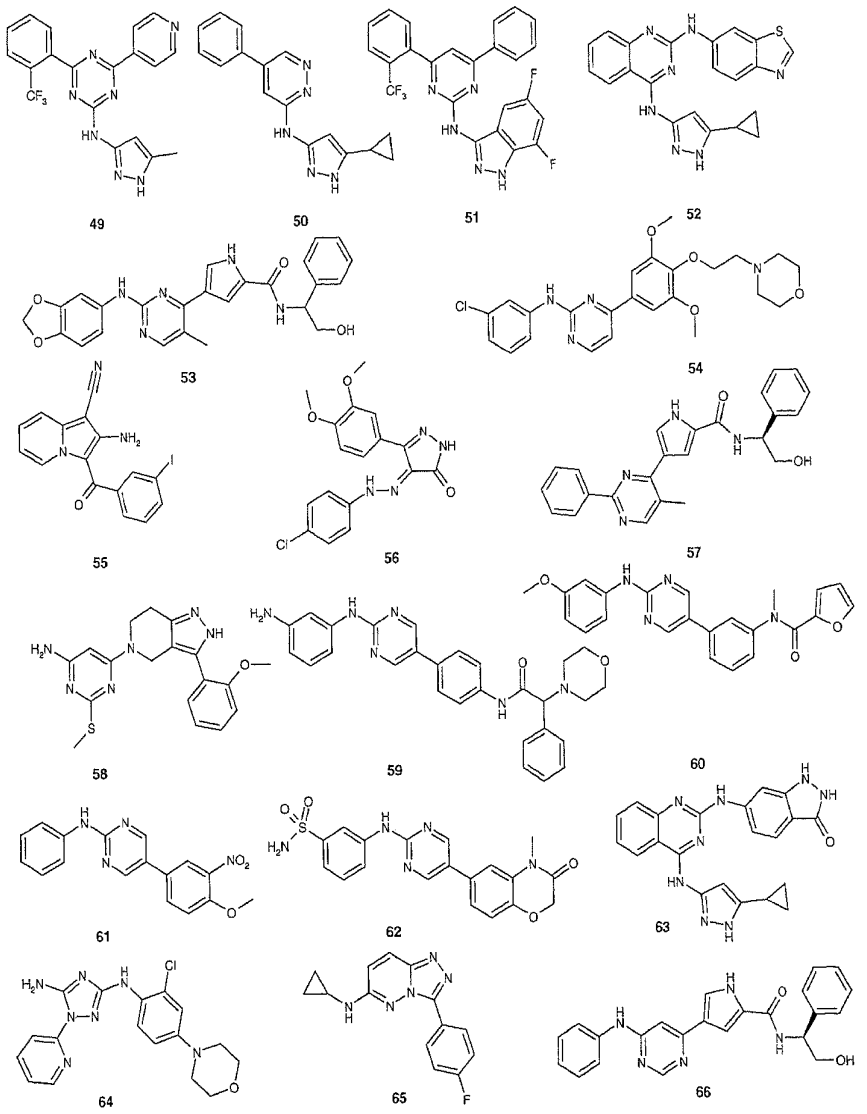


Figure 11. CDK inhibitors from Vertex.

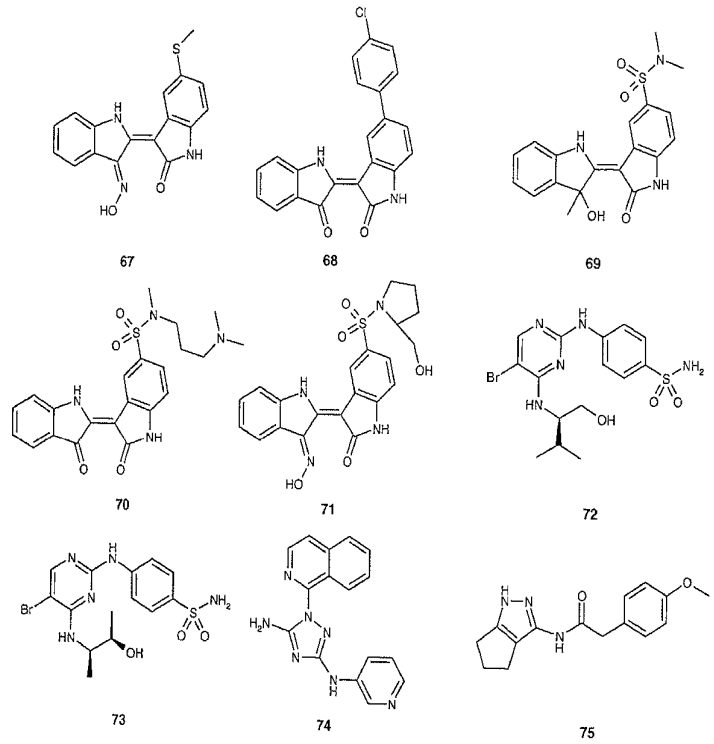


Figure 12. CDK inhibitors from Schering AG.

as CDKs and GSK-3β inhibitors, which are useful for the treatment of cancer but also against autoimmune, cardiovascular and CNS diseases. Inhibition of MCF-7, H460, HCT116 and DU145 was determined. The specified compound 67 exhibited IC_{50} values of ~ 1 μM against all four human tumour cell lines. CDK2/cyclin E, CDK1/cyclin B and CDK4/cyclin D1 kinase inhibitory activity was also determined: seven compounds had IC_{50} values of 0.02 – 5 μM against CDK2, with compound 67 being the most potent. An example of the compounds claimed in [198] as CDKs and GSK inhibitors is given by 68 (IC_{50} against CDK2 = 0.030 μM; inhibition of MCF-7 cell proliferation at 0.5 μM). Novel indirubin derivatives are claimed in [199] to be inhibitors of GSK-3β, CDK1, CDK2, CDK4, CDK5, CDK6, CDK7, CDK8 and CDK9 and to be useful for the treatment of cancer, autoimmune, cardiovascular, CNS diseases and infection. A specified compound exhibited an IC_{50} value of 70 nM against CDK2/cyclin E and inhibition of MCF-7 cell proliferation at 300 nM (69). A total of 67 compounds are claimed in a subsequent patent application [200] as CDK1-9 and GSK-3β inhibitors with improved solubility, selectivity and effectiveness. The IC_{50} values for CDK2 and MCF-7 inhibition ranged 10 – 100 and 30 – 600 nM, respectively, the specified compound 70 being the more potent.

The use of indirubin derivatives as vascular endothelial growth factor receptor (VEGFR) and CDK2/cyclin E inhibitors was disclosed in a patent application [201]. The IC_{50} values against VEGFR and CDK2/E ranged 50 – 200 and 20 – 2500 nM, respectively (50 and 90 nM for compound 71).

2,4-Diamino pyrimidine is a common substructure for compounds claimed in [202,204] as CDKs and GSK-3β inhibitors. More than 500 compounds are exemplified with characterisation data in [202]. A total of 57 compounds out of the

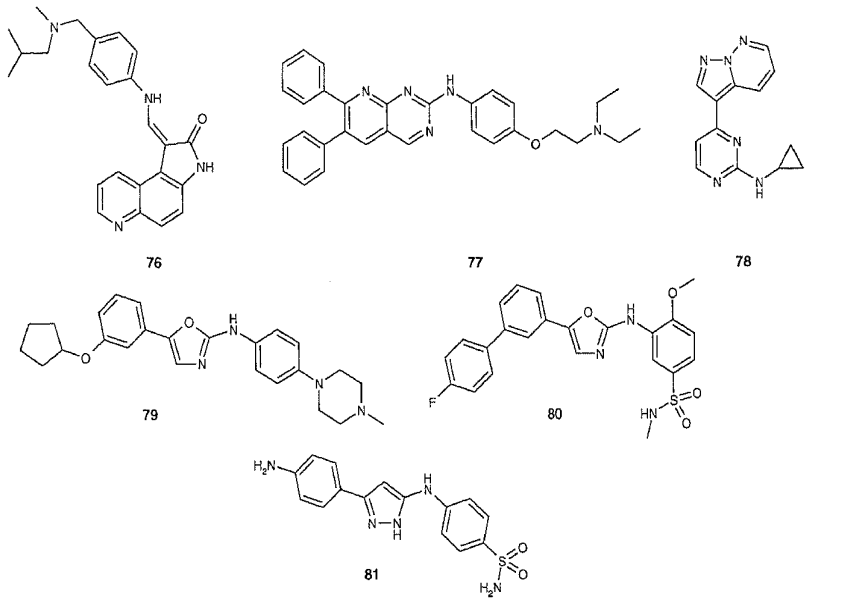


Figure 13. CDK inhibitors from Glaxo and SKB (GSK).

68 tested showed IC_{50} values of 4 – 1000 nM against CDK2. An example is provided by pyrimidine 72 (IC_{50} against CDK2: 4 nM; inhibition of MCF-7, H460, HCT116 and of DU145 cell proliferation between 0.2 and 0.5 µM). Over 100 related 2,4-diaminopyrimidine of type 73 are reported in [204] with similar inhibitory activities against CDK2 and MCF-7 cell line. Some novel pyrimidine and thiophene intermediates are also claimed in this patent.

Only 20 2,5-diaminotriazoles, such as compound 74, are disclosed in a patent application [203] as CDKs, GSK-3β and VEGFR inhibitors. Biological data are presented for the inhibition of CDK2 and VEGFR2 and of MCF-7 cell proliferation (IC_{50} values of 0.2, 0.02 and 0.7 µM, respectively, for the specified compound 74).

Finally, 64 tetrahydrocyclopentapyrazole derivatives (e.g., 75) are claimed to be CDK1-9 and GSK inhibitors, some of them at low micromolar concentrations, against, for example, CDK2/cyclin E [205].

2.8 Glaxo and SKB

2.8.1 Glaxo

A total of 15 compounds of type 76 (Figure 13) are disclosed in a patent application [206] as CDK inhibitors and are targeted particularly against CDK4 and/or CDK2. Biological data are presented for the inhibition of CDK4/cyclin D and CDK2/cyclin E (IC_{50} values of 0.1 and < 1.0 µM, respectively, for the specified compound 76). All of the compounds are exemplified by syntheses, with characterisation by ^{1}H-NMR and MS data.

2.8.2 SKB

Amino-heterocyclic derivatives are claimed in a patent as Myt1, Weel, Tie2 and CSBP/p38 kinases inhibitors [207]. Activities of the five specifically claimed compounds (e.g., 77, Figure 13) against CDK1 and CDK2 were also maintained, but no specific biological data were presented.

A patent focused on fused pyrazine derivatives CDK inhibitors subsequently appeared [208]. A specified compound is 78 (IC_{50} against CDK4 and CDK2 < 0.1 and < 1.0 µM, respectively).

2-Amino-oxazole is a common substructure for compounds claimed in [209,210]. No specific biological data are reported in [209] for the inhibition of VEGFR2, CDK4 and CDK2. A total of 49 tested compounds are stated to have IC_{50} values in a broad range (< 0.1 to < 10 µM) for the inhibition of the three kinases (0.1 µM for the specified

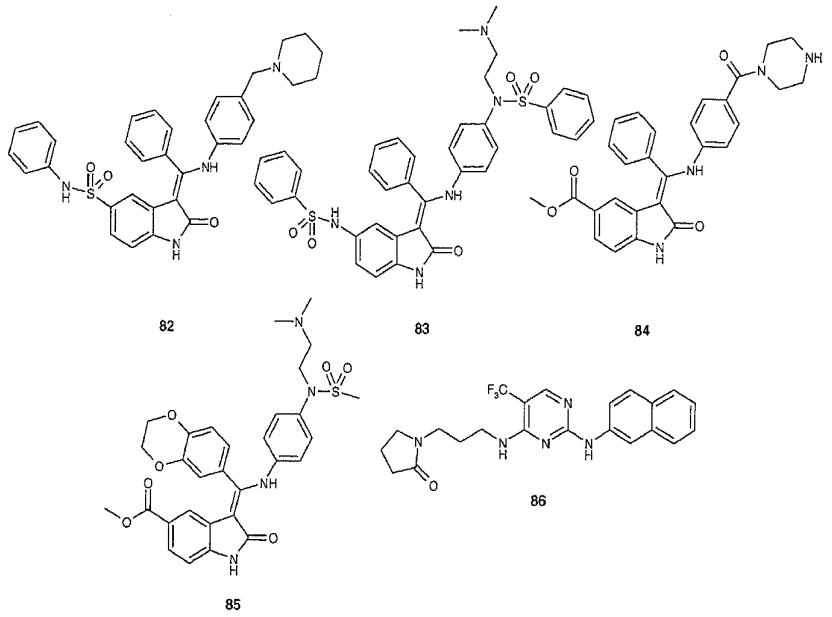


Figure 14. CDK inhibitors from Boehringer-Ingelheim.

compound 79) [209]. Compounds of [210] (e.g., 80) are claimed to be particularly effective at inhibiting CDK2 and/or CDK4 and/or VEGFR2 at concentrations that range 0.0001 – 1 µM displaying specificity relative to other kinases. Over 230 compounds, including intermediates, are exemplified by syntheses, with characterisation by ^{1}H-NMR and MS data.

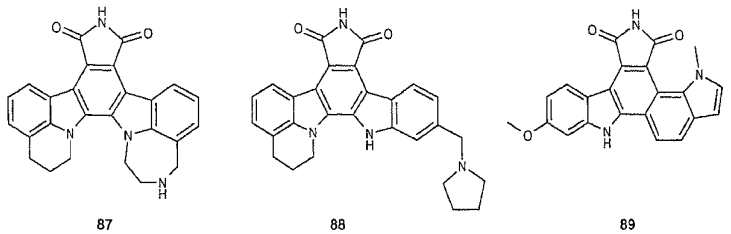
About 15 anilinopyrazoles of type 81 are specifically claimed in a focused patent application on CDK2 inhibitors [211]. Several compounds are stated to be nanomolar inhibitors of CDK2 (pIC_{50} against CDK2 = 9.0 – 9.99 for the specified compound 81) as published in a subsequent paper [19].

2.9 Boehringer Ingelheim

A total of 284 novel 5-substituted indolinone derivatives, such as compound 82 (Figure 14), are claimed in a patent application [212]. These compounds are stated to have inhibitory effects on CDKs. The ability of 14 compounds to inhibit proliferation of SK-UT-1B leiomyosarcoma tumour cells was determined *in vitro*, with IC_{50} values ranging 0.9 – 85 nM. Assays for measuring the inhibition of CDKs/cyclins are described but no data are provided. Related aminomethylidene indolinone derivatives, such as compounds 83, 84 and 85, are claimed in three patents [213,214,216] as generic protein kinase inhibitors, which encompass both serine/threonine kinases (including CDKs) and receptor and non-receptor tyrosine kinases (including VEGF, EGF and IGF or c-Src), revealing a trend towards the development of a kinase platform. An *in vivo* experiment for determining the effect of the compounds on mice carrying implanted or injected tumour cells is described in [213], but no resultant data are presented. A trend towards a multikinase characterisation is also visible in a further patent in which novel 2,4-diamino-substituted pyrimidine derivatives of type 86 are claimed to be inhibitors of several protein kinases, including CDKs, Src, PLK and Aurora2 [215]. Over 900 compounds are exemplified and > 100 of them are stated to have IC_{50} values < 0.1 µM against CDK1/cyclin B1, but no specific biological data are presented.

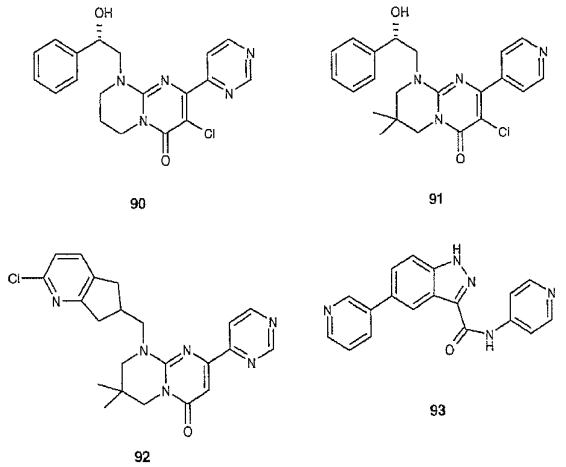
2.10 Eli Lilly

Eli Lilly based its work in the CDK inhibition field around Staurosporin-related indolocarbazoles [20]. Three patent applications were filed throughout 2001 and 2002 dealing with novel indolocarbazoles with different aryl groups replacing one of the two indole moieties, and endowed with CDK4 inhibitory activity [217-219].



87 88 89

Figure 15. CDK inhibitors from Eli Lilly.



90 91 92 93

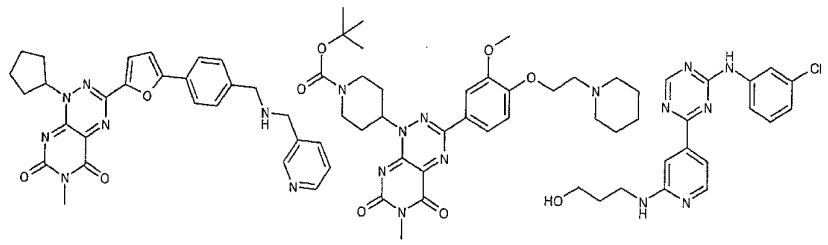
Figure 16. CDK inhibitors from Sanofi-Synthelabo.

Reference [217] deals with novel ^{1}H-pyrrole-2,5-dione derivatives like compound 87 (Figure 15). The compounds are stated to be CDK4 inhibitors, useful for the treatment of a wide range of cell proliferative disorders. The ability of nine compounds to inhibit CDK4 activity was demonstrated *in vitro* using specific protein substrates but no specified biological data are presented.

A total of 277 related indole derivatives like compound 88 are claimed in [218]. The CDK4/cyclin D1 inhibitory activity of these compounds was investigated: the IC_{50} values ranged 0.037 – 1.56 and 0.011 – 0.811 µM when, respectively, RbING peptide and Rb21 protein were used as a substrate. A further 103 compounds based on the indolo[6,7-a]pyrrolo[3,4-c]carbazole scaffold were disclosed in [219]. Indolocarbazole 89 is one of five compounds specifically claimed in the patent (IC_{50} against CDK4/cyclin D1 = 43 and 2 nM using RbING and Rb21 substrates, respectively; inhibition of HCT116 and H460 cell proliferation is present at 1.8 and 1.09 µM respectively). Values for the inhibition of Rb phosphorylation are also given for the specified compound (90 – 97% inhibition at its IC_{50} concentration) [219]. Compounds disclosed in the three patents are all exemplified by syntheses, with characterisation by both ^{1}H-NMR and MS data.

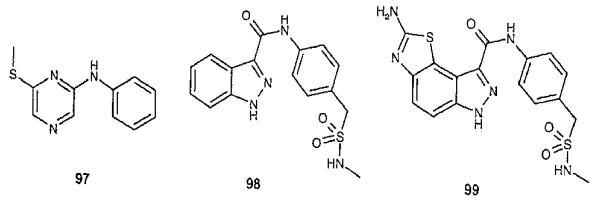
2.11 Sanofi-Synthelabo

4-Pyrimidone is a common substructure for compounds such as 90, 91 and 92 (Figure 16) claimed in [220-222] as GSK-3β alone or GSK-3β and TPK2 (CDK5/p25) inhibitors. Compounds disclosed in these patents are stated to show IC_{50}



94 95 96

Figure 17. CDK inhibitors from Janssen.



97 98 99

Figure 18. CDK inhibitors from Astex Tech.

values in the range 5 nM to 2 µM and 2 nM to 5 µM when tested in GSK-3β and TPK2 (CDK5/p25) inhibition assays, respectively. No specific data are provided in [223] where novel indazole-3-carboxamide derivatives (e.g., 93, Figure 16) are claimed as CDK1, CDK2 and CDK4 inhibitors with an IC_{50} value of < 20 µM against the three enzymes.

2.12 Janssen Pharmaceutica

Three patents were disclosed by Janssen in the CDK area in 2004 [224-226]. As with other companies they contain evidence of a kinase platform effort, because several kinases with relevance in cancer and other therapeutic areas are descibed.

Novel 3-furanyl and 3-phenyl derivatives of toxoflavine [21], active as kinase inhibitors, and a process for their preparation are claimed in the first two patent applications [224,225]. The compounds are stated to inhibit CDKs, kinases and phosphatases involved in cell cycle regulation (e.g., tyrosine kinases such as Wee1, Mik1 and Myt1, and tyrosine dephosphatases such as CDC25 and Pyp3), and they were evaluated for their inhibitory activity against CDK4, AKT3 and CDC25B in SPA, filter and fluorogenic assays, respectively. The specified compounds displayed in the respective assays, pIC_{50} values of 7.041, 7.51 and 7.83 (94, [224]; Figure 17) and of 6.75, 6.843 and 7.661 (95, [225]). Moreover, the compounds are stated to exhibit improved water solubility over toxoflavine while retaining their antiproliferative activity [224,225].

Only six compounds are exemplified in a patent focused on 2-amino-4-aryltriazine [226] active as CDK1, CDK2, GSK-3, VEGFR or EGFR2 kinases inhibitors. The specified compound 96 has IC_{50} values of 16 nM and 2.56 µM against CDK1 and VEGFR, respectively, and it is one of the two derivatives for which selectivity data over a further 12 kinases are presented (IC_{50} against GSK-3 = 17 nM; and against CK1 = 1.41 µM; inactive against EGFR1, PKA, PKCγ, PKCβ2, CK2, calmodulin kinase, ERK-2, PDGFR and IRK; inhibition of Hela, HCT-116, and A375 cell proliferation are reported at 105, 48 and 80 nM, respectively). The specified compound 96 also increased the survival time by 12 days when tested *in vivo* in mice bearing A375 xenograft (daily dosing with 125 or 150 mg/kg i.p.).

2.13 Astex Technology

Astex is focusing its activity on families of proteins implicated in human diseases, such as cancer, CNS and inflammatory diseases, including kinases, proteases and phosphatases. As part of this programme, three patents have been disclosed in the CDK field [227-229] throughout 2004. A total of 40 novel pyrazine derivatives, such as compound 97

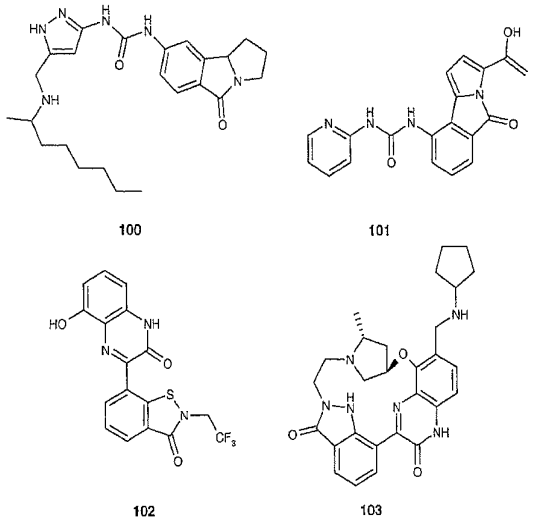


Figure 19. CDK inhibitors from Banyu Pharm.

(Figure 18) are specifically claimed as CDK inhibitors in [227]. They are stated to have IC_{50} values in the 3 – 147 μM range against CDK2.

Indazole 3-carboxamide is a common substructure for compounds such as 98 and 99 claimed in [228,229] as CDK inhibitors, with activities against CDK2 in the micromolar range (no specific data are provided). Interestingly, two oral formulations are disclosed in the latter two patents [228,229].

2.14 Banyu Pharmaceuticals

Banyu has focused its activity in the CDKs arena mainly on CDK4 and CDK6, as shown by patent applications filed [230-234] and papers published [22] during the last few years.

Biarylurea is the common substructure for compounds such as **100**, and **101** disclosed in [230-232] as potent CDK4 and CDK6 inhibitors (Figure 19; **100**: IC_{50} against CDK4/cyclin D1 and CDK4/cyclin D2 = 61 and 19 nM, respectively [230]; **101**: IC_{50} against CDK4/cyclin D1, CDK4/cyclin D2 and CDK6/cyclin D2 = 61, 19 and 13 nM, respectively; inhibition of HCT116 and MKN-1 tumour cells proliferation at 13 and 100 nM, respectively [231]).

Novel fused pyrazinone derivatives, such as compounds **102** and **103** (Figure 19,) are claimed in [233,234] to be potent CDK4 and CDK6 inhibitors, many of them in the low nanomolar range (compound **102**: IC_{50} against CDK4/cyclin D2 = 1 nM).

A common feature in all these patents is that chemistry is detailed and compounds are all characterised by both ^{1}H-NMR and MS data.

2.15 Albany Molecular Research

Novel heterocycle substituted purine derivatives are stated to be potent CDKs inhibitors [235,236]. The ability of the compounds to inhibit the growth of solid tumours was measured in athymic mice after i.p. administration. The specified compound **104** (Figure 20) produced delays in tumour growth of 2.5, 2.9 and 1.4 days, at 15, 10 or 6.7 mg/kg (q4dx3), respectively, compared with vehicle-treated controls [235]. Several compounds of [236] are stated to inhibit growth of BT-579, MCF7 and numerous other transformed cell lines with a GI_{50} value of < 0.01 μM. Novel azabenzothiopyranoindazole derivatives were claimed in another patent application [237] as CDK inhibitors. The *in vitro* inhibitory activities of > 20 compounds were determined in Hela S-3 cells selected for growth on plastic using the method of Skehan *et al* [23]. Compound **105** had a GI_{50} value of 0.1 μM.

2.16 Amgen

Patents diversified across several templates, but all of them containing the thiazole ring, were disclosed by Amgen in the last few years. Thiazolyl-substituted quinolinones are claimed to be inhibitors of cell proliferation or apoptosis [238] and to be

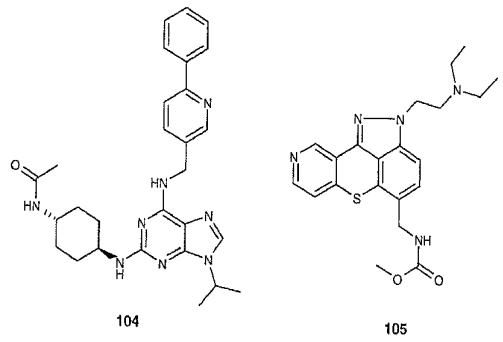


Figure 20. CDK inhibitors from Albany Molecular Research.

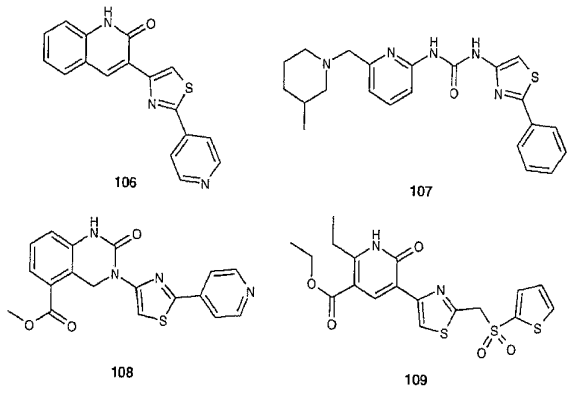
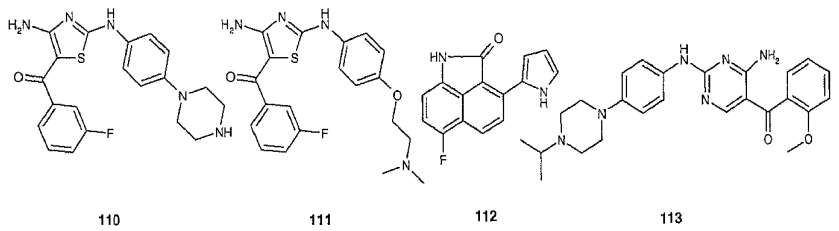


Figure 21. CDK inhibitors from Amgen.

endowed with serine-threonine kinases inhibitory activity. Compound **106** (Figure 21) showed IC_{50} value of < 1 μM against CDK2 and CDK5/p25. Novel thiazolyl ureas such as compound **107**, useful for the treatment of cancer and neurological disorders, are claimed in a patent application [239]. The compounds are stated to be inhibitors of apoptosis and CDK/cyclin kinases (IC_{50} of < 0.5 μM against CDK2 and CDK5/p25) and of GSK activity. Compounds targeted at CDK2 and CDK5 were also disclosed in two subsequent patent applications [240,241]. Tetrahydroquinazoline **108** (Figure 21) is reported in [240] with an IC_{50} value of < 1 μM against CDK2 and CDK5/p25 and it is stated to inhibit cell proliferation of human PC-3, HCT116 or HT-29 tumour cell lines with IC_{50} value of < 5 μM. An example of the compounds described in [241] is the 2-oxopyridine derivative **109** (IC_{50} against CDK2 and CDK5/p25 = < 0.5 μM; inhibition of PC-3, HCT116 and HT29 cells proliferation with IC_{50} of < 1 μM).

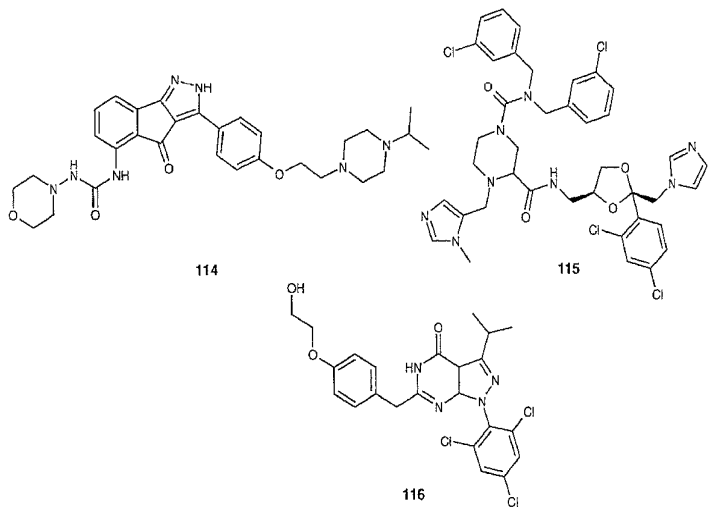
2.17 Hoffmann-La Roche

Novel diaminothiazole derivatives are claimed in a patent application as CDK inhibitors [242], and are particularly active against CDK4, CDK1 and CDK2 (IC_{50} values of 0.013, 0.3 and 0.2 μM, respectively for the specified compound **110**, Figure 22). As a follow-up of the same main class expansion, > 140 novel 4-amino-2-[(4-alkoxy-aryl)amino]-5-benzoyl thiazoles have also been disclosed [243] as inhibitors of CDK4,



110 111 112 113

Figure 22. CDK inhibitors from Hoffman-La Roche.



114 115 116

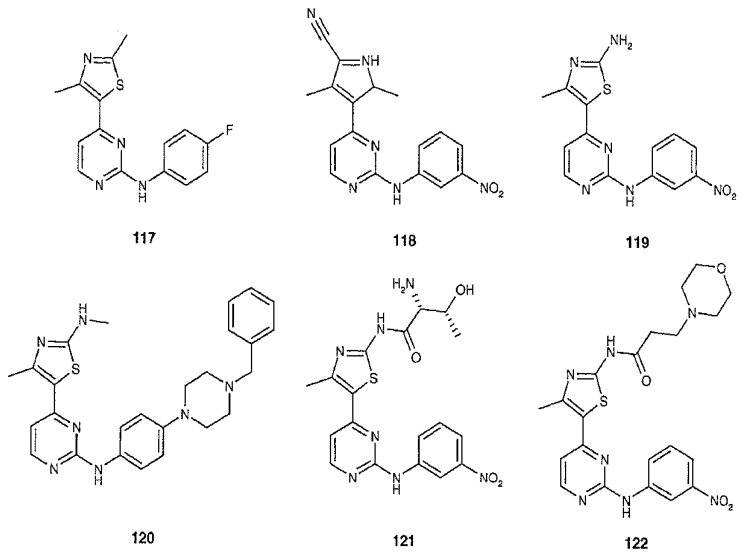
Figure 23. CDK inhibitors from GPC Biotech.

selective over CDK2 and CDK1 (e.g., 111: IC_{50} against CDK4, CDK2 and CDK1 = 0.032, 0.892 and 0.126 µM, respectively). Solid-phase chemistry is detailed in this patent and compounds are characterised by MS (ES) [243]. Novel naphthostyril compounds such as 112, primarily targeted at CDK2 are claimed in [244]. Finally, a patent focused on CDK inhibitors for the treatment of solid tumours has been disclosed [245]. More than 350 compounds, exemplified by syntheses and characterised by MS (ES) data, are claimed to be CDK4 inhibitors. Compound 113 is reported in the patent with IC_{50} values of 0.171, 3.56 and 10 µM against CDK4, CDK1 and CDK2, respectively.

2.18 GPC Biothecnology

Indeno pyrazoles such as 114 (Figure 23) are specifically claimed to be CDK inhibitors in a patent application [246] (IC_{50} of < 0.01 µM against CDK2/cyclin E, CDK2/cyclin A CDK4/cyclin D1 and CDK6/cyclin D2, and 0.1 µM against Cdc2/B for the specified compound). Along the same lines indenopyrazole derivatives were disclosed in a subsequent patent application [247] as preferential CDK inhibitors.

Cytotoxic antifungal activity, including CDK-activating kinase 1 (CAK1) *N*-myristoyltransferase and prenyltransfrease inhibitory activity is claimed for novel heterocyclic compounds of type 115 [248].



117 118 119 120 121 122

Figure 24. CDK inhibitors from Cyclacel.

Compounds such as 116 (Figure 23) were disclosed [247] as CDKs inhibitors. Several assays are described and used to demonstrate the biological activity and utility of the compounds. Biological data are presented for certain compounds of [247]; for example, compound 116 showed IC_{50} < 0.1 µM against CDK2/cyclin E and CDK1/cyclin B, IC_{50} < 1 µM against CDK4/cyclin D1; HCT116 viability < 0.1 µM and IC_{50} < 0.01 µM in an anti-HIV assay.

2.19 Cyclacel

Among the smaller companies, Cyclacel has been one of the most active in the field of CDK inhibition, being one of the first to produce a clinical candidate (*R*-roscovitine; CYC-202, Figure 1). A number of patents claiming combinations of a CDK inhibitor with established anticancer agents (with doxorubicin [249], mitoxantrone [250], cisplatin [251], docetaxel [252] and gemcitabine [253]) have therefore been published in 2003 – 2004. A second cluster of patents deals with the so-called CYC-400 series [24,25], basically 2-anilino-4-(heteroaryl)pyrimidine compounds. These patents differentiate mainly for the nature of the 4-heteroaryl moiety: in a first application [254] a 2,5-disubstituted-1,3-thiazol-5-yl ring is present and yields compounds like 117 (Figure 24) with an IC_{50} value against CDK2/cyclin E of 19 nM and micromolar activity against a panel of tumour cell lines. A second patent case [255] refers to 4-(1H-pyrrol-3-yl) heteroaryls (e.g., 118) with a good potency against both the enzyme (IC_{50} against CDK2/cyclin E of 30 nM) and in cells (IC_{50} for antitumour proliferation using a standard 72h-MTT assay of 0.40, 0.26, 0.31, 0.26 and 0.74 µM, against, respectively, A549, HeLa, HT-29, MCF-7 and Saos-2 cells). A selection of compounds featuring a 4-(2-amino-1,3-thiazol-5-yl) heteroaryl moiety is presented in [256]: compound 119 is a subnanomolar inhibitor of CDK2/cyclin E (IC_{50} = 0.2 nM) and a submicromolar inhibitor of tumour cells (IC_{50} for A549, HT-29 and Saos-2 cell of, respectively, 0.22, 0.34 and 0.42 µM). The last two patent cases [257,258] report further elaborations on the 4-heteroaryl moiety. Compounds with a modulated profile of CDK inhibition are reported. As from Table 1, compound 120 (Figure 24) acts mainly as a CDK7/cyclin H and CDK4/cyclin D1 inhibitor, while compound 121 is a preferential CDK9/cyclin T1 inhibitor, and 122 is a larger-spectrum CDK inhibitor.

These patents exemplify well the trend towards an increased appreciation of the importance of CDK7/cyclin H and CDK9/cyclin T1 as further targets for antitumour activity.

2.20 Other companies

Several other companies have claimed many structural types of CDK inhibitors, and their use as antitumour agents or in other therapeutic areas. Most of them will be reviewed in this section.

2.20.1 Bayer Corporation

A total of 375 novel aminopyrimidine derivatives, such as compound 123, were disclosed in a patent application as

Table 1. (IC_{50} values in μM).

Compound	CDK1/cyclin B	CDK2/cyclin A	CDK2/cyclin E	CDK4/cyclin D1	CDK7/cyclin H	CDK9/cyclin T1
120	2.4	2.2	0.26	0.0098	0.019	1.1
121	5.4	0.85	0.13	2.0	0.34	0.070
122	0.24	0.098	0.0025	1.7	0.20	0.11

CDK: Cyclin-dependent kinase.

CDK inhibitors [259]. Biological data are presented for eight compounds such as inhibition of HCT116 human cell proliferation (IC_{50} = 0.24 – 3.56 μM). An *in vivo* assay in *nu/nu* mice with HCT116, H460 or A549 xenografts is also described. It is stated that the specified compound (123, Figure 25) shows antitumour activity in HCT116 and H460 xenografts but no data are presented.

2.20.2 Cellular Genomics

Compound 124 (Figure 25) is an example of novel imidazo[1,2-a]pyrazine derivatives claimed as modulators of a variety of protein kinases, including CDKs (no data presented), particularly for the treatment of cancer and also in combination with a chemotherapeutic or a radiotherapeutic agent [260].

2.20.3 Cytopia

A kinase effort not limited to CDK inhibition is visible in a patent application [261] disclosing novel pyrazine derivatives like 125 (Figure 25). The compounds are stated to be useful for treating conditions involving serine-threonine kinases (e.g., CDKs, ERK2, c-Jun, p38 MAPK, PKA and PKC), receptor tyrosine kinases (e.g., EGF, HER2, IR, CSFIR, C-kit, Kdr and Flk-4), and cellular tyrosine kinases (e.g., Src, Frk, Btk, Csk, Abl, Fak, Hck, JAK1-3 and TYK2).

2.20.4 Daiichi Pharmaceuticals

A total of 211 fused aromatic compounds were disclosed in a patent application focused on CDK4 inhibitors useful for the treatment of cancers [262]. An example is given by compound 126 (Figure 25); IC_{50} value against CDK4 and CDK2 is 0.096 and 1.0 μg/ml, respectively; inhibition of HCT116 cell proliferation is also reported with a GI_{50} value of 88 ng/ml.

2.20.5 Enzon

Polymeric acyl derivatives of indole like the conjugate of alsterpaullone (127; Figure 25) were disclosed as CDK inhibitors [263].

2.20.6 Kyowa Hakko

CDK2 inhibitors were disclosed in a patent application dealing with novel indoe derivatives [264]. An example is given by compound 128 (Figure 25; IC_{50} against CDK2 = 0.96 μM; inhibition of Saos-2 and U2OS cells proliferation at 0.3 and 0.68 μM, respectively).

2.20.7 LG Biomedical

Compounds having a phenolic core structure are claimed to be kinase inhibitors, encompassing CDKs [265]. Compound 129 (Figure 25) is reported in the patent with an IC_{50} value < 0.5 μM for CDK2 and CDK5. The compound was also tested *in vitro* against 61 tumour cell lines ($logGI_{50}$ values from -5.1 to -8.0).

2.20.8 LG Chem Investment

Two patents focused on CDKs inhibitors were disclosed [266,267]. Compound 130 (Figure 25) is one of the 47 3-hydroxychromen-4-one derivatives specifically claimed in [266] (IC_{50} against CDK2 and CDK4 = 0.185 and 0.195 μM, respectively). The oral toxicity of these compounds was also investigated in ICR male mice (LD_{50} value > 3000 mg/kg for the specified compound). Compounds of [267] were tested against CDK2 and CDK4; for example, compound 131 (IC_{50} values against CDK2 and CDK4 = < 0.05 and < 10 μM, respectively). The compounds exemplified in the two patents are all characterised by ^{1}H-NMR and MS data.

2.20.9 Merck & Co.

During the last few years Merck activity in the kinase field has been focused mainly on tyrosine kinases and, among serine-threonine kinases, on Akt. Compounds such as 132 (Figure 25) generically claimed in a patent application as kinase inhibitors [268], were also tested in a CDK2 and CDK4 assay but appear to be selective KDR inhibitors [26]

2.20.10 Natrogen Therapeutics

Natura (compound 133, Figure 25) is the only compound specifically claimed in a patent application from Natrogen Therapeutics [269]. This agent is stated to act through modulation of CDKs activity (particularly CDK4/6 and CDK2) and to strongly suppress cyclin D-mediated CDK4/6 activity. The compounds are also stated to have minimal toxicity and side effects, compared with the prior art molecules, and a better chemotherapeutic index due to improved solubility and bioavailability.

2.20.11 Nicholas Piramal India

Novel flavone derivatives endowed with CDKs inhibitory activity were disclosed in a patent application [270]. The compounds are stated to be particularly CDK2/cyclin E and CDK4/cyclin D1 inhibitors with greater selectivity towards

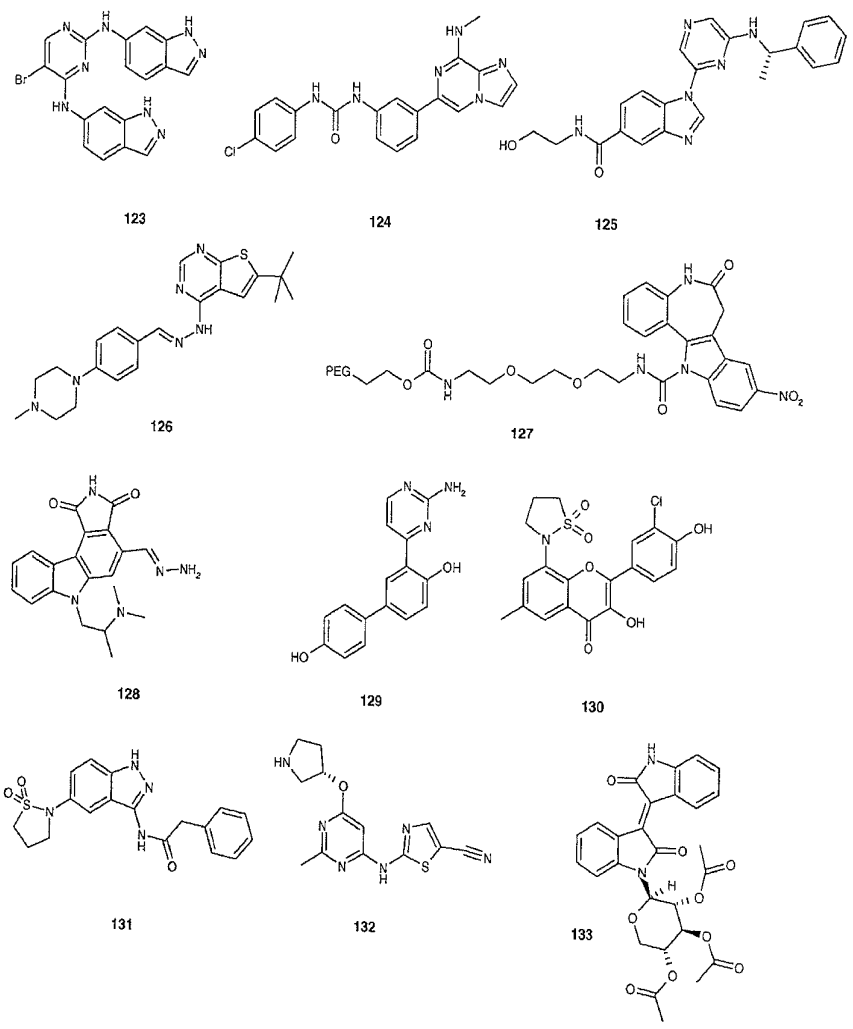


Figure 25. CDK inhibitors from other companies (1).

CDK4/cyclin D1. Compound 134 (Figure 26) is reported to have an IC_{50} value of 0.08 μM against CDK4/cyclin D1 and of 6.0 μM against CDK2/cyclin E. *In vitro* antiproliferative activity was also determined against several cell lines.

2.20.12 Ortho-McNeil Pharmaceutical

CDKs (particularly CDK1, CDK2 and CDK4) and protein tyrosine kinases (particularly VEGFR2 and EGFR2) inhibitors were disclosed in a patent dealing with novel triazole

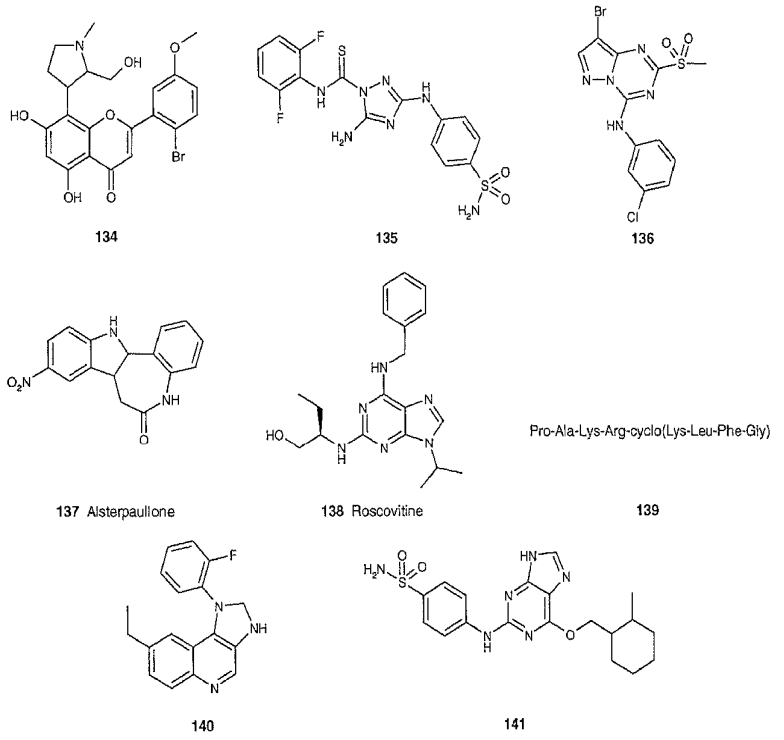


Figure 26. CDK inhibitors from other companies (2).

derivatives, such as 135 (Figure 26; IC_{50} value of 0.0006 µM against CDK1) [271]. A protocol to determine the antitumour activity of the compounds *in vivo* is also described, but no resultant data are presented.

2.20.13 SCRAS

A total of 31 pyrazolo[1,3-a][1,3,5]triazine derivatives are claimed as CDK and GSK-3 inhibitors [272]. Several compounds, including 136 (Figure 26), were tested for their CDK1/cyclin B activity and all of them demonstrated higher antiproliferative activity than Roscovitine in Mia-PaCa2 cells, although no specific biological data support the claim.

2.20.14 Yamanouchi Pharmaceutical

Two known CDK inhibitors, asterpaullone (137) and roscovitine (138, Figure 26) were disclosed for use in novel medicinal compositions which suppress the production of β-amyloid [273], thus being useful for the treatment of Alzheimer's disease.

2.20.15 Novartis Pharmaceuticals

Small cyclic peptides, such as compound 139 (Figure 26), were claimed to be inhibitors of E2F-1 binding to cyclin A (IC_{50} = 1 nM for the specified compound) [274].

^{1}H-Imidazo[4,5-c]quinoline derivatives, such as compound 140, were disclosed for use in the treatment of protein kinase-dependent diseases [275]. The compounds were tested for the inhibition of several kinases, including CDK1 (IC_{50} = < 0.5 µM for the specified compound).

2.20.16 Cancer Research Campaign Technology

A patent dealing with novel purine derivatives, such as compound 141, active as CDK inhibitors, was filed in 2002 [276].

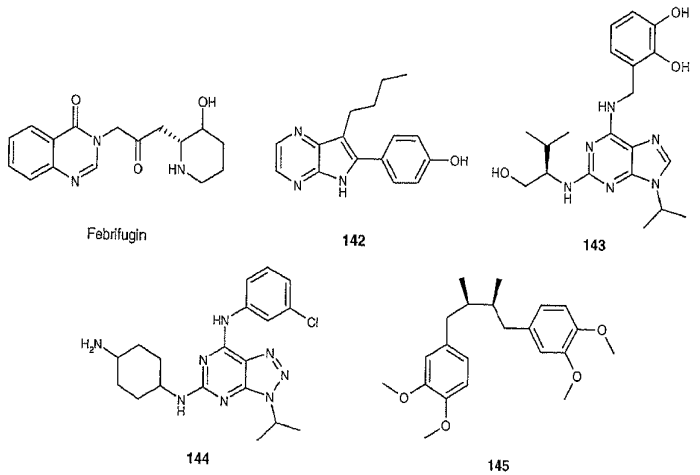


Figure 27. CDK inhibitors from public and academic institutions.

The specified compound displayed IC_{50} values of 6 and 4 nM against CDK1 and CDK2, respectively.

2.20.17 Welgene

Novel large circular target-specific antisense regions complementary to one or more target RNAs expressed from target genes are claimed in a patent application [277]. The large circular antisense molecule is used to treat any human disease in which modulation of gene expression can be beneficial to intervene in the disease initiation and progression. Thus, large circular nucleic acid were constructed employing a phagemid vector and the M13KO7 helper bacteriophages containing the antisense sequence for CDK2, TNF-α, NF-κB, c-myc, c-myb and c-Ki-ras. Antisense to CDK2 were added to the HeLa cervical cancer cell line antisense HeLa transfectants displayed > 70% inhibition of tumour growth at an antisense concentration of 2 nM [277].

3. Academic institutions

CDK inhibition has also been the subject of an intense patent activity from public and academic institutions which can be divided into two main topics.

3.1 New uses and combinations of known compounds

New uses and combination of known compounds are described in several patents; for example, modulation of specific CDKs, such as CDK9, is described in [278]. This patent deals specifically with CDK9 modulators in the context of cardiovascular diseases, such as cardiac hypertrophy. Flavopiridol is quoted as a CDK9/Cyclin T1 inhibitor to be used alone or in combination with known cardiovascular agents, such as ACE inhibitors.

Febrifugine (Figure 27) analogues are inhibitors of an unusual CDK claimed in [279]; inhibition in the micromolar range is reported for some derivatives against the recombinant plasmodial CDK (PfMRK) with relevance in the setting of malarial diseases. Again in the anti-infective field, flavopiridol is claimed as a tight binder to the transcription elongation factor (P-TEFb), dramatically inhibiting its activity. As P-TEFb is required for HIV propagation and replication, flavopiridol, alone or in combination with known anti-HIV agents, can be used as a treatment of HIV infections and AIDS [280]. More generally, CDK inhibitors such as roscovitine, purvalanol or flavopiridol are claimed for inhibiting the replication of a drug-resistant pathogenic agent [281]. Promotion of apoptosis in cancer cells by co-administering CDK inhibitiors and cellular differentiation agents is described in [282]. Mentioned CDK inhibitors are flavopiridol, UCN-01, roscovitine, olomoucine and butyrolactone, and histone deacetylase inhibitors (e.g., tricostatin A), protein kinase C (PKC) activators (e.g., bryostatin) or retinoids are claimed for the co-administration. Data supporting combinations and uses of imatinib (Glivec®, Novartis) with CDK inhibitors (e.g., flavopiridol) were published in [283]. Three patents related to the use of indirubin, hymenialdisine and paullone derivatives were issued by the Centre National de la Recherche Scientifique (CNRS) of France. A thorough disclosure of biological data is

made reporting biochemical, structural and cellular data for several compounds [284-286].

3.2 New compound classes

A series of pyrrolopyrazines dubbed aloisines [27] were disclosed from the CNRS with aloisine A (142, Figure 27) being the most potent compound, with an IC_{50} value for CDK1/cyclin B, CDK2/cyclin A, CDK2/cyclin E and CDK5/p35 of 0.15, 0.12, 0.40 and 0.16 µM, respectively [287]. Compound 143 (Figure 27) is the most potent (IC_{50} vs. CDK1/cyclin B = 0.25 µM) of a series of 64 purine analogues claimed in a 2001 patent [288]. A related patent concerns azapurine derivatives, such as compound 144 [289]. Compounds are claimed as CDK1 – 4 and 6 – 8 inhibitors as well as inhibitors of PLKs and GSKs. Potent cellular activity in MCF-7 and K562 cells is reported for compound 144 with IC_{50} values of 6.1 and 7.4 nM, respectively. Indole-3-carbinoles, naturally-occurring components of *Brassica* species vegetables (i.e., cabbage and broccoli), are stated to be useful for the treatment of metastatic cancer breast acting through CDK6 inhibition, although no biological data are reported [290]. Finally, derivatives of norhydroguaiaretic acid, such as compound 145, are inhibitors of CDK1/cyclin B, blocking cell cycle and inducing apoptosis at double-digit micromolar concentrations [291].

4. Expert opinion

There are several trends emerging from a survey of the recent literature on CDK inhibition:

- Selective CDK2 and/or CDK4 inhibition have been the major goals of industrial research teams in the last few years, but the concept of multi-CDK inhibition (e.g., CDK1/CDK2/CDK4) has gained popularity due to recent findings in basic biology, hinting that selective CDK4 or CDK2 abrogation may not be sufficient to counteract in vitro proliferation, at least within certain cellular contexts.
  CDK2 remains the main target in oncology as shown by the biological parts of the patents that invariably describe CDK2/cyclin A and E assays.
  CDK1 is not normally seen as a primary target but nevertheless its inhibition should reinforce the antitumour activity of a CDK2 or CDK4 inhibitor.
  CDK4-selective inhibitors are not usually claimed in the patent literature 2001 – 2004. CDK4 inhibition has been recently perceived as a mechanism that tumour cells can easily bypass in order to proliferate and that may be restricted to pRb-negative tumours. However, recent literature point towards new roles and functions of CDK4 that may account, in part, for the preclinical activity or CDK4/6 inhibitors [28]. CDK5 has also been a relatively common target for the CNS therapeutic area although a clinical proof of concept is still lacking. A trend towards a better understanding and an increased appreciation of CDK7 and CDK9 inhibition in the cancer setting is also observable (e.g., enzymes are quoted in the most recent patents), although published data are still scanty.
- The concept of combining a cell cycle inhibitor and specifically a CDK inhibitor with either classical cytotoxics or targeted compounds is theroretically sound. Combination with classical cytotoxics relies upon the hope that CDK inhibitors may be able of recruiting cells into a more sensitive phase of the cell cycle (e.g., G1/S). Combination with molecular targeted compounds, such as VEGFR inhibitors, combines different mechanisms, such as cell cycle block with antiangiogenic activity, thus coupling two different but potentially synergistic mechanisms. Consequently, patent coverage of combinations of CDK inhibitors with other antitumour drugs continue to be pursued by several companies and academic institutions.
- Although most patents dealing with CDK inhibition still indicate oncology as the main target therapeutic area, a trend can be observed towards extending their use in other disease settings with the support of experimental findings. In particular, cardiovascular, antiviral and proliferative glomerular disease are the most quoted spin-off indications.
- A discernable trend towards including CDK inhibition in the context of a broader kinase effort is also evident in the recent patent literature. Several patents comprise a list of validated or potential kinase targets and often parallel kinase data are reported in the biological sections.

In general, CDK inhibition may be considered a rather mature field of research, with some companies having worked for > 10 years around this topic. However, a proof of concept in the clinical setting is still awaited. In the meantime, several new compounds are entering clinical trials, mainly in oncology, and over the next few years light will be shed revealing the real impact of CDK inhibitors on human health.

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